AEROMICROBIOLOGY: AN ASSESSMENT OF A NEW MEAT RESEARCH COMPLEX

by

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Introduction

With few exceptions, microorganisms can be found everywhere. Air is one place where these microbes can be found and is considered as a major vehicle in the transmission of them from one environment to another. The microbiology of the atmosphere has been known as early as the time when the dispute of spontaneous generation occurred.

Due to the fact that microorganisms can be noxious to human, animal, and plant; microbial quality of air is becoming more and more important and has been emerged to the field of air pollution studies. Not only the airborne microorganisms but also other airborne particles that have allergenic effect such as pollen are included in these studies.

Airborne microorganisms in food processing plants are extremely important due to the economic and health problems they may cause. Controlling the sources of microbial contamination in food processing plants has a great influence on lowering the chance of producing harmful food products.

Several methods can be used in evaluating the microbial quality of air. However, no single method or device that can enumerate all microbial populations with different physical characteristics.

The objectives of this study were 1) to monitor microbiological quality of air in a new meat processing laboratory before and after occupancy, 2) to study the microbial profile of air in the new laboratory, 3) to monitor

the effect of temperature, humidity, number of people and their activity on the microbial populations in the new meat processing complex, and 4) to study the microbiological profile before, during, and after the slaughtering operation in the slaughtering room.

Literature Review

The Presence of Microorganisms in Air and Their Sources. Air contains gases, water droplets, and microscopic and submicroscopic particles of pollen, dust, and microorganisms (bacteria, molds, yeasts, and viruses). These microorganisms cannot grow or multiply because of the absence of nutrients, but they can be transmitted in air for long distances. Depending upon the density, different microbes are found at different heights from the ground (Banwart, 1979). Location and environmental conditions (humidity, temperature, density of human populations and their activity) of the samples taken have a big influence on the amount and kinds of microbial populations in air (Kingsley, 1967). Some microorganisms such as Micrococcus and Sarcina, Gram-positive rods, and fungal spores were found at an altitude of 3,000 m in the air mass above the North Atlantic (Gregory, 1973). An increased wind speed, which assists in dehydrating and breaking up larger droplets, results in increased numbers of small particles including microorganisms. The spread of airborne microbes, therefore, could be reduced by blocking the places from which most microorganisms are emitted to air. Studies of the 1967-1968 foot-and-mouth disease epidemic in England showed that some microorganisms were found more than 60 km away from the main epidemic area (Hugh-Jones and Wright, 1970).

The major sources of airborne microbes include human beings (through sneezing, coughing, talking, etc.), animals,

vegetation, sewage, and dust particles that act as adsorbents of microbes and endotoxins.

Sneezing ejects droplets, which may contain viruses and bacteria, into the air. The big droplets suspend in air for a short time, whereas the small ones (<2 mm) stay a longer time and travel longer distances. Sneezing could deposit as many as 10^6 particles of various sizes and types (Joklik et al., 1988). When air turbine equipment is used, the breathing zone of a dentist can be contaminated with saliva, bacteria, and other particles that may exist in the patient's mouth (Dimmick and Akers, 1969). Fecal wastes and other discharges from human activities are the most important sources of indirect air contamination.

Animals contribute to the aerobiological pollution directly by creating air turbulence while moving in the field or indirectly by their fecal materials and other discharges. High numbers of bacteria (10^{10} and 10^{11}) Colony Forming Unit (CFU)/gram were found in poultry litter. Gram negative, coryneform bacteria, and micrococci were the predominant groups. By the action of wind, rain splash, and the movement of birds and workers, a high portion of these microorganisms are discharged into the air (Jones et al., 1984). Birds also carry bacteria on their feathers and deposit them into the atmosphere at many sites in a processing plant. Flying birds and insects are also responsible for the transmission of

microorganisms from one place to another. Movement of flying animals and insects helps in redistribution of airborne microbial populations. The emission of some pathogens to air from an affected animal can occur by breathing, sneezing, or by splash from falling infective urine or feces (Hugh-Jones and Wright, 1970).

Activated sludge, trickling filter, and rock filter systems used in sewage treatment plants create aerosols and droplets containing microbes (including pathogens), which enter the environment. As many as 10^5 bacteria can be found in one m^3 of air surrounding sewage treatment plant. However, this number decreases dramatically as the distance from the plant increases (Edmonds, 1979). A study by Miller et al. (1977) showed that Aspergillus fumigatus was detected frequently in the atmosphere surrounding a sewage sludge compost site. This microorganism is believed to cause asthmatic spasms, fever, and malaise (Edmonds, 1979). Total count, coliform count, fecal coliform number, and fecal streptococci count are frequently used as indicators of the air quality surrounding sewage treatment plants (Lembke and Kingsley, 1985).

The action of wind, dry leaves, grass, and twigs contribute large numbers of plant-associated microorganisms to the air on dry days. When leaves and twigs become wet and the wind speed is lower, the number of microbes discharged

into the air declines (Lindemann and Upper, 1985). A large number of fungal spores, $10^3/m^3$, was found in the air inside farm buildings at the time of hay shaking. These microbes and the associated airborne dust have a big effect on the workers' health (Kotimaa et al., 1978).

Water mass is not as significant as land mass in emitting microorganisms and small particles into the air. A study of the micropopulations at 690 meter height indicated that a water mass discharged a lower number of microorganisms into the air than a land mass (Fulton and Mitchell, 1966). This finding confirmed the hypothesis presented by Junge (1964) and Gregory (1964), who argued that the sea acts as a sink in cleaning the lower layers of the atmosphere.

Water used as a coolant in cooling towers associated with nuclear and fossil-fuel fired plants is another significant source of airborne micropopulations (Lighthart and Frisch, 1976). It has been found that as many as 10^{10} viable bacteria/second could be emitted to the air from a 15-meter-high cooling tower (Lighthart and Frisch, 1976).

Airborne Dust and Endotoxin. Exposure to one or more of the organic dust components, which include bacteria, viruses, yeasts, molds, rickettsiae, hair, etc., may produce allergic disease such as Farmer's Lounge, Bird Breeder's, Cheese Washer's and several other diseases (Cole, 1983). Cases of hypersensitivity pneumonitis (allergic inflammation of the

lung) have been found among workers involved in raising or processing turkeys (Boyer et al., 1974).

Endotoxin, the lipopolysaccharide found in the cell wall of gram-negative bacteria, is a very important element in organic dust particles. Inhalation of endotoxin could cause hypersensitivity diseases. When lipopolysaccharides are swallowed, they may cause diarrhea in susceptible people (Pernis et al., 1961). More than 40,000 poultry workers in the U.S.A. are exposed daily to high concentrations of biological aerosols (Cole, 1983).

Endotoxins ranging from 4.5 to 48 μg/gram of airborne dust have been detected in samples taken from swine and poultry confinement units (Thedell et al., 1980). Lower amounts of endotoxin were found in poultry processing plants (Olenchock et al., 1982). A comparative study between two turkey confinement units showed that the units supplied with a spray system for aerosol control had lower mortality rate among turkeys. This was due to a lower amount of airborne organic dust in the sprayed unit (Cravens et al., 1981). Clark et al. (1983) showed that even though the airborne dust quantities were not significantly different, the airborne concentrations of endotoxin in a poultry house were much higher than in a swine farm. That was attributed to the difference between the respirable-size portions (26% in the swine farm and 40% in the poultry house) of the particles containing the endotoxin.

Table (1) shows the endotoxin content of various organic dusts in different industrial establishments that house different types of activity.

Table 1: Comparison of Endotoxin Content of Various Organic Dusts and Other Materials.

Source of Sample[1]	Endotoxin (μg/mg)	
	Range	Median
Swine C.U.[2]	0.01 - 0.10	0.03
Poultry C.U.	0.10 - 0.18	0.12
Poultry C.U.(settled dust)	0.008- 0.20	0.04
Cattle barn (hay feed)	0.44	0.44
Cattle barn (hay feed) (settle dust)	0.98	0.98
Cotton card room	0.23 - 1.60	0.78
Compost plants	0.002- 0.10	0.008
Compost plant (exterior air)	0.001- 0.21	0.05
Compost plant (bulk)	0.01 - 0.20	0.04
Heat dried sludge	0.01 - 0.20	0.10

[1] Unless noted all samples are of inside air.
[2] C.U. = Confinement Units.
{Adopted from the work of Clark et al. (1983)}.

Sources of Atmospheric Microbial Contamination in Food Processing Plants. In studying human beings as a source of airborne microorganisms, Heldman and Hedrich (1971) placed human beings in an enclosed area and monitored the airborne populations in the space. Sneezing, talking, laughing, falling hair, using soiled laboratory coats, as well as shedding from hands and arms contributed a high number of microorganisms to the atmosphere (York, 1973). Heldman and Hedrick (1971) cited Sunga's (1968) report, indicating that human beings shed between 3.3×10^3 and 7.2×10^3 microbes per min into air. It has been established that the microbial flora shed from the human skin influences the work environment. For example, in some dairy plants, many of the microorganisms found on the workers' skin were also found in the processing plant atmosphere (Wilkinson, 1966).

Figure 1 shows a summary of airborne bacterial count in an isolated space above floor drains of a dairy plant. When flooding was continuous, the microorganisms were washed away instantly. On the other hand, when water was allowed to pool for a longer time, the microbes had a chance to reproduce, which resulted in higher microbial number in the space adjacent to the drain (Heldman and Hedrick, 1971).

The air turbulence created by a ventilation system deposits some microorganisms into the air. As the ventilation system continues to operate, the airborne microbes decrease

as a result of the relative depletion of microorganisms in the space. Figure 2 is a diagram illustrating the behavior of the total microbial count in air before, during, and after operating a ventilation system (Heldman and Hedrick, 1971).

The raw materials in processing plants, whether they are live animals or birds, fruits or vegetables, contribute a major part of the microbial air contamination. The microbes are deposited into the air during handling and processing of the raw materials. The high number of microorganisms in the first stages of food processing confirm the influence of raw materials on the microbial count in air (York, 1973; Kotula and Emswiler-Rose, 1988).

The Mechanisms of Airborne Particles Deposition. As a general concept, the atmosphere from the ground upward consists of four layers: laminar boundary layer, turbulent boundary layer, outer friction layer, and convection layer. The thickness of each layer is affected by weather conditions, such as sunlight and cloudiness. When sun rays penetrate the air to the ground surface, parts of them are absorbed and other parts are reflected. The upward/reflected rays heat up the air layers near the ground. The outcome from this phenomenon is an unstable condition in which the less dense air (near the ground) tends to go up, and the more dense air (far from the ground) falls downward. This circulating aerosystem is known as a convectional current,

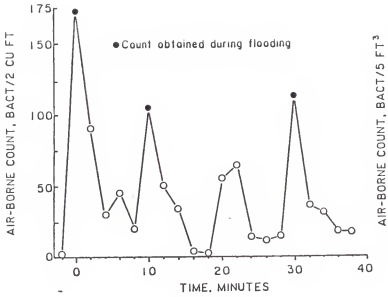


Figure 1:

Effect of Flooding at 10-min Intervals on Airborne Bacteria Counts in Isolated Space Above Drains in Four Food Plant Areas. Airborne Microorganisms.

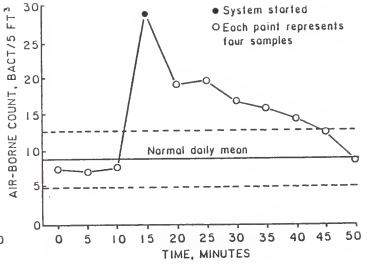


Figure 2:

Contribution of Ventilation System to The Population of

(Heldman and Hedrick, 1971)

which helps deposit some microorganisms and minute particles into the air (Polunin, 1961). Brownian motion in air is also known as one of the physical attributes that help in suspending tiny particles (including microorganisms). As in liquids, the airborne particles are in constant collision with air molecules, some of which possess sufficient energy to move the particles. Moving objects make the effect of this phenomenon more efficient (Dimmick and Akers, 1969).

The pattern of particle deposition in an enclosed place is affected by factors such as shape, size of the particles, surface texture, the design of the building, and air velocity. The indoor air resembles that found in a stirred settling chamber in which the moving objects stir or mix the small particles including microbes with air. Ventilation also causes some of the take-off action of microbes as well (Cox, 1987). The number of microbes in air tends to decrease in the enclosed environment, if not continuously renewed by the introduction of fresh air to replace the dead microorganisms or those deposited on the walls, ceiling, and floor.

The Importance of Airborne Microbes. Microorganisms in air or anywhere else can be harmful or beneficial, depending upon the way they affect our daily lives.

The fact that airborne pathogens are not frequently found in the outdoor air could be attributed to physical agents, such as ultraviolet rays. Indoor air, however, may contain pathogens contributed by both human and animal occupants. The

situation is more obvious in urban areas, where people spend most of their time indoors (Joklik et al., 1988). Edmonds (1979) reported that there are 63 airborne human, animal, and plant diseases caused by various types of molds, yeast, bacteria, and viruses.

Air in hospitals, with their natural activity of serving some patients carrying infectious diseases, support the spread of airborne pathogens. Tuberculosis and respiratory tract infection are common examples of airborne infection found in hospitals. The appearance of Pseudomonas and Staphylococcus is critical, especially in burn units where the skin of burned patients is very susceptible to microbial invasion. Common microbes found in hospitals usually become resistant to the frequently used antimicrobial agents so that any infection may become difficult to overcome (Cox, 1987; Lindemann and Upper, 1985; Gregory, 1973).

Several very important animal diseases such as foot-and-mouth disease, are caused by living airborne entities. About 16 animal diseases (mostly viral) caused by airborne microorganisms were reported to cause economic losses in livestock (Edmonds, 1979).

About 19 plant diseases (mostly fungal) were reported by USDA in 1965. Between 1950 and 1960, economic losses caused by airborne microbes on plants ranged from $150 to $500 million annually (Edmonds, 1979).

The Survival of Microorganisms in Air. The survival of microorganisms in air depends on physicochemical and climatic characteristics (such as humidity, gases, and temperature) and on their ability to change to spores. A general classification of survival power in the aerosol state consists of two main groups: 1) hardy microbes, including plant pollen, many fungi, spore forming bacteria, and encysted protozoa, and 2) tender pathogens, which commonly spread among individuals in close proximity, including inhaled pathogens (Gregory, 1973). Physical agents such as UV and other types of short-wave rays, relative humidity (<60%), and low temperature decrease the viability of most microorganisms (Edmonds, 1979). A study to investigate the survival of Escherichia coli revealed that the rapid death of the aerosolized microbes was mostly at low relative humidity (<50%) and between 15 and 30 C, with half-lives of 14 or 3 min, respectively (Washes et al., 1986). The difference in the total viable count between up and downwind from a sewage treatment plant was relatively small in most samples. Coliform counts downwind, however, showed much higher numbers than the ones obtained upwind (Adams and Spendlove, 1970). This finding suggests that the presence of coliform in air requires a continuous supply, whereas gram positive microorganisms have the ability to survive a longer time under the same conditions.

The death of vegetative gram negative bacteria could occur 1) in a high range of relative humidity (R.H.) in which

the ribonucleic acid-protein complex dissociates and is subjected to subsequent ribonuclease degradation or inactivation of protein synthesis, or 2) at low RH in which oxidation to some of the cell component occurs (Hess, 1965). By an unknown mechanism, carbon monoxide (one of the most dangerous air pollutants) may penetrate and protect spores of Bacillus subtilus var. niger at low RH (Lighthart, 1973).

Methods of Air Sampling. The main reason for sampling air is to evaluate the air quality and get some information about the hygienic condition in a particular location. About 1000 years ago, an evaluation of a place for a health clinic was made in Baghdad, Iraq. Several pieces of fresh meat were used to determine the air quality in different places in Baghdad for the purpose of choosing the best site to build a clinic. The place in which the last piece of the meat spoiled was chosen to build the clinic (Ministry of Education, 1982).

There are two main methods by which airborne microbes can be sampled: 1) collection into a liquid (buffer solution or liquid medium) and 2) collection onto solid and semi-liquid media or filters. Conditions such as particle size, wind speed, and direction influence the efficiency of airborne particle collection.

Many devices have been in use for aspirating airborne particles; each one has different advantages and limitations (Edmonds, 1979).

An agreement upon the problems involved in sampling airborne microorganisms was reached at the International Aerobiology Symposium where it was stated:

> 1) That sampling, as now conducted, is essentially an art, 2) That each investigator must, of necessity, employ the sampling procedure yielding the most productive and useful information for his purpose, 3) That the loss of viability incurred as a result of the sampling step is difficult to assess and may not always be constant, 4) That concerning respiratory diseases, the animal host is the ultimate sampler, although the animal can not always be utilized for this purpose especially in studies relating only to survival of airborne microorganisms, and 5) That data obtained with any specialized sampler should be correlated with at least some results obtained in a similar manner with a standard reference sampler. The sampler chosen should be one that is widely used and readily available. (Brachman et al., 1964)

Gravitation settling is one of the oldest means used to study airborne organisms. A gravity slide or petri dish with suitable medium is exposed horizontally to the air to receive the settling particles. The exposure time can vary from 15 min to several hours, depending on the information desired. This method is not very effective in collecting quantitative data, but acceptable when qualitative results are sought (Gregory, 1973; Kingsley, 1967). The Gravity Settling Plate (GSP) method was compared with Anderse's sequential volumetric air sampler (Andersen, 1958) in estimating airborne bacteria. A much lower number of viable particles was detected with GSP than with the Andersen sampler. The most important difference was that the GSP method showed falsely negative results,

especially with some potential pathogens such as coagulase-positive Staphylococcus aureus (William et al., 1972).

Filtration of air is an effective way to capture most airborne microbes passing through a filter. The air can be sucked through different fibrous or porous media. The filters are designed with various pore diameters to impact a specific size of airborne particles. The captured microbial cells can be washed and inoculated onto a suitable medium or direct microscopic examination of impact microorganisms can be used. For microscopic examination, the filter should have a smooth surface such as a molecular membrane (Edmonds, 1979). The molecular filter membrane, which is charged with microbes on the upper surface, can be placed on a suitable nutrient agar. Filtration is more quantitative than the other methods. One drawback of this method is inconsistent viable counts of some microorganisms. A study conducted at the Naval Biological Laboratory using five different filter materials showed that the spores of Bacillus globigii can be recovered immediately after collection or as long as 72 hours later. The vegetative cell, however, could not be recovered even with immediate inoculation (Dimmick and Akers, 1969). This result indicates that the recovery of vegetative cells from filters is questionable.

Electrostatic precipitation is suitable for small particles and large volumes of air. The principle of the system is that as the air passes through an electrostatic

sampler, microorganisms are charged near the entrance and then attracted to an electrode of opposite charge (Gregory, 1973; Kingsley, 1967).

In the Liquid Trap method, air to be examined is drawn through a liquid, which could be a buffer solution or a nutritional medium. As the air is sucked through the liquid, the aerobiological particles are retained. The system has been recommended for sampling delicate microorganisms to avoid drying and subsequent death (Cox, 1987; Edmonds, 1979).

The all-glass impinger (AGI-30, Figure 3) is a standardized device used in airborne microbe sampling. It has been chosen as a reference sampler for comparison in development of new air samplers (Brachman et al., 1964). Air is drawn through a curved tube to simulate the nasal passage and through a jet, which is 30 mm from the impinger base. The end of the tube has a smaller diameter, which helps impinge the airborne particles at high velocity. The distance between the tube end and the impinger can be manipulated in favor of collecting different sizes of airborne particles (Cox, 1987; Dimmick and Akers, 1969).

Direct deposition (impaction) on a semi-solid surface is a very effective method for sampling microbes in air.

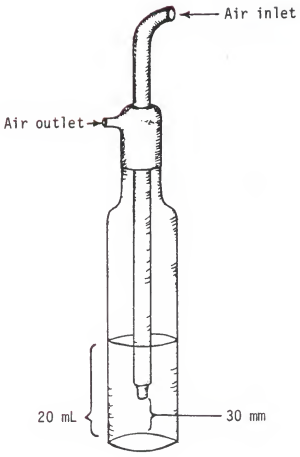


Figure 3:

AGI-30 impinger.

(Cox, 1987)

Using the principle of sucking air onto a semi-solid medium, many instruments have been developed to improve the efficiency of capturing as many airborne microbes as possible. The different devices, including Andersen Sampler, Slit Sampler, Drum Sampler, Multi Jet Monitor, and the newly developed Surface Air Sampler have different sucking rates and physical capabilities for collecting various sizes of airborne microorganisms. The name and the characteristics of the instrument, therefore, must be reported whenever used in air quality monitoring (Cox, 1987; Edmonds, 1979; Kingsley, 1967).

It has been proven that inertial force is responsible for impacting particles from an air stream. When air is rapidly sucked by applying negative pressure through a tapered jet and the velocity imparted to a particle is sufficient, the particle inertia will overcome the aerodynamic drag. The particle, therefore, will cross the air stream (Figure 4) and move toward the impaction surface (Cox, 1987; Andersen, 1958). Different sizes of airborne particles under the same conditions of pressure and jet-exit surface distance will not be trapped equally as a result of having different velocities or different sizes.

A cascade impactor is widely used in air quality monitoring. It is based on the Inertial Force Theory. It has been used extensively in the field of industrial hygiene

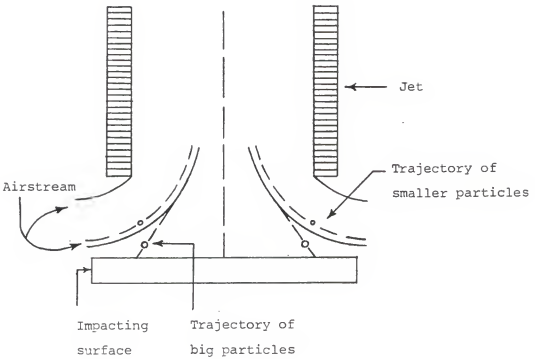


Figure 4:

Trajectories of different sizes.

(Cox, 1987)

for the sizing of solid and liquid aerosols. Sampling with a cascade impactor allows the estimation of the entire size distribution. This sampler consists of four stages that operate at 1.4 L/min (Jones et al., 1983). The last stage has a paper filter for a high collection efficiency of submicron particles (Mercer, 1965).

The Andersen sampler (Andersen, 1958) is another system that collects airborne particles in several sizes and densities. It consists of six stages (Figure 5). The size of the holes become smaller for each succeeding stage. The velocity of air is equal on each plane, but increases in each succeeding plane. The changes of the velocity from one stage to another help in separating smaller sizes and densities as the air stream goes downward through the six stages. The hazardous sizes (0.2 - 5 microns) of airborne particles are collected in stages three to six and particles > 5 microns (nonhazardous) are retained on the first and second stages. A comparative study by Jones et al. (1985) showed that the number of viable particles collected onto a single culture plate was only half that collected on the six stage Andersen Sampler. The use of the Andersen sampler with one of the six stages containing culture, however, indicated no statistical difference.

Impaction of airborne particles with a Slit Sampler (Bourdillon et al., 1941) involves direction of a measured volume of air against a rotating nutrient agar plate.

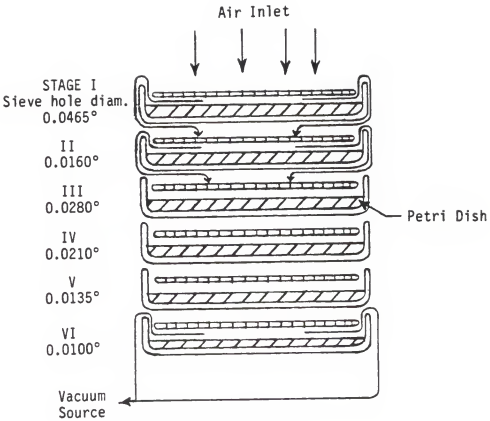


Figure 5:

Diagram of 6-stages Andersen Sampler.

(Andersen, 1958)

The Slit Sampler is more effective in monitoring indoor air, but not suitable for outdoors especially in a windy period (Gregory, 1973). Several factors including slit-to-agar distance, slit width, and the air velocity were found to affect the sampling efficiency (Goldberg and Shechmeister , 1951).

Centrifugal Samplers have long been used in industrial hygiene as filters for removing dust. Centrifuging of air sucked into the system results in a circular path of airborne particles, thereby increasing their effective mass, which will help deposit the particles on the walls. For removing fine particles, a smaller size of the centrifugal device and a higher velocity is required. The most common method used for sampling airborne microorganisms is the returned flow system (Figure 6). When the viable particles are sucked and circulated, they are carried to the walls of the sampler. Suitable fluid is injected to form a thin layer on the inside wall. This fluid helps carry the deposited microbes into the bottom (Erriwgton and Powell, 1969; Cox, 1987).

Airborne Microorganism Populations in Food Processing Plants. The quality and wholesomeness of all food products are affected not only by the quality of the initial materials, the cleanability of the processing equipment, and the packaging materials, but also by the purity of the

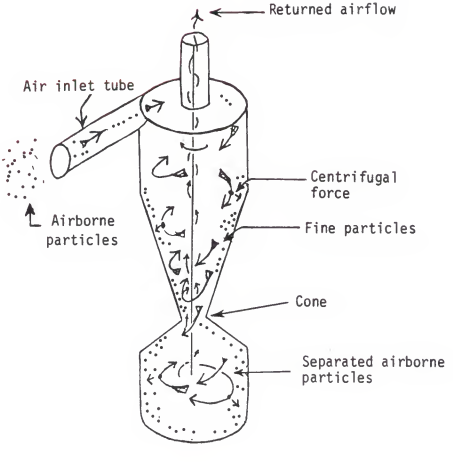


Figure 6:
Centrifugal Sampler.

(Cox, 1987)

atmosphere surrounding the processing area. The processed food can be contaminated directly either by personnel or the processing equipment, or contaminated with airborne microorganisms. The appearance of microbes in the atmosphere of a food processing plant means that: 1) these microorganisms are coming from the food being processed, and/or 2) if they come from another source, they will go to the food. The outcome in either case is not desirable.

The post-pasteurization contamination sources in dairy processing plants must be eliminated in order to have extended shelf-life of the final product (Angevine, 1959). Airborne bacteriophage has been reported to be the main cause of single-strain culture failure. By using multi- bacterial strains and rotating cultures, the problem of phage in dairy plants can be minimized. The presence of bacteriophage was first discovered by: 1) a collection into sterile water, 2) exposure of dishes containing sterile skim milk for 10 min, and 3) exposure of inoculated agar plates with a specific strain (Whitehead and Hunter, 1945).

The most critical point in the line of milk pasteurization plants, as far as airborne microbes are concerned, is the packaging area. The microbial populations in this area have the best chance to invade the processed milk (Sunga et al., 1966). In a microbial study of this area in a milk processing plant, a correlation of $r = 0.86$ was found between the number of airborne microorganisms and the number of

microorganisms that contaminated the product (Radmore et al., 1988).

In poultry processing plants, airborne microbial populations are extremely important in relation to salmonellosis. The microbial load in a poultry processing plant atmosphere is mainly attributed to the live birds (Clark and Lenz, 1969). An evaluation of microbial air quality in two poultry plants indicated that air of the shackling and dressing rooms were more contaminated with psychrophilic bacteria than any other places in the plant, and the highest number of yeasts was found in the air surrounding the dressing operation. The density of airborne molds, however, was not significantly different among the processing rooms. This which indicates that the molds could be attributed to another sources of contamination rather than processed birds (Kotula and Kinner, 1964). It is well known among poultry processors that the stress that birds suffer before slaughtering affects the amount of flapping and fecal materials released during hanging. These actions undoubtedly contribute to the high microbial loads in the air of the early stages of the processing line. Outbreaks of food-borne diseases caused by Salmonella are often traced back to consumption of contaminated poultry or poultry products. About 12 Salmonella serotypes were recovered in a comparative study among seven turkey processing plants. The majority of these serotypes

were recovered from the air in the hanging area (Zottola et al., 1970).

Since the "appearance" of new, emerging pathogens (Campylobacter jejuni and other pathogenic bacteria), federal agencies have focused on the factors (such as air) in meat processing plants that may contribute to the total count of microorganisms in or on meat products (Stern, 1981). During slaughtering and further processing, the movement of the equipment as well as workers might cause the release of some microorganisms on the animals' skin. These microorganisms will settle down and could contaminate the meat products (Kotula and Emswiler-Rose, 1988).

Contamination from airborne microbes may vary among different types of food processing plants. For instance, the airborne microbial contamination in a pork facility (Kotula and Emswiler-Rose, 1988) was higher by about 10 times than that in dairy plants.

Control of Airborne Microorganisms in Food Processing Plants. The control of airborne microorganisms in the outdoor environment is not an easy task compared with control of the indoor environment. In a closed environment, many physical and/or chemical agents can be applied to reduce the total number of airborne microbes. The only thing that can be helpful in reducing the microbial load outdoors is control of organic materials. Natural agents such as UV light, humidity, temperature, and wind direction and speed have a big influence

on the total number of airborne microbes in the outdoor atmosphere.

The most positive approach in controlling airborne contaminants indoors is to remove all contamination sources from the space where the food undergoing processing might be exposed to air. Packaging operations require the most pure air.

The contamination sources, which include the ventilation system, floor drains, shedding by human beings, and transport openings could be controlled in several ways. 1) In ventilation systems, the air filters differ in their design according to the job they will accomplish. The performance efficiency of filters relies on the fiber diameters. The efficiency of retaining a high number of particles can be increased by using filters having a smaller diameter and by decreasing porosity. Medium-efficiency filters may be used as pre-filters to remove large particles. To be effective, the air filter must be located as close as possible to the point where airborne contaminants may occur, so that the contaminants are removed before the air enters an enclosed space. 2) Eliminating floor drains as a source of contamination requires having a good design for the surfaces that prevents solid collection, which will facilitate water flow into the drain. The drains also could be sanitized periodically to reduce the contamination level in flooding. 3) The use of clean-room clothing, head covering, masks, and gloves eliminate the emission of micro-

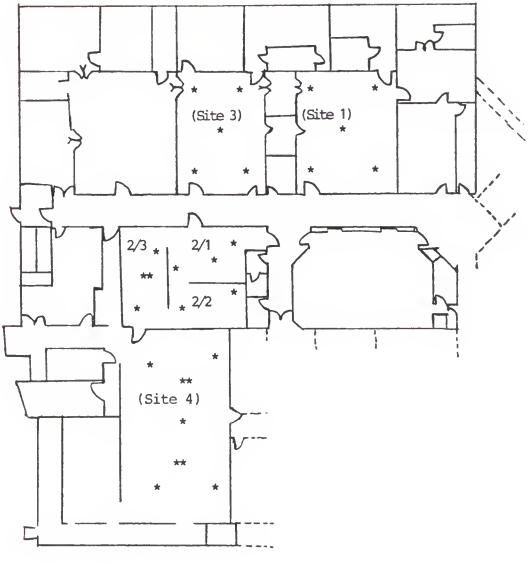
organisms into the processing environment. A good hygiene training program for personnel will effectively help reduce the contamination coming from the workers. 4) Openings between the rooms are sources of microbial contamination, but could be minimized by keeping the ventilation rates low. Having a higher ventilation rate in a less contaminated room than in a highly contaminated one will significantly reduce the transport of microbes to the less contaminated room (Heldman and Hedrick, 1971).

In conclusion, much has been written about microorganisms in air; however, more needs to be studied in terms of understanding their environmental impact.

Materials and Methods

This study was conducted in the new meat laboratory complex at Weber Hall of the Department of Animal Sciences and Industry, Kansas State University. The sampling operation started in March, 1988 and terminated in April, 1989. The laboratory complex (Figure 7) consists of three main rooms, three coolers, two freezers, a smoke house, and slaughtering room. The air was sampled at the designated sites which include Site 1, 2/1, 2/2, 2/3, 3, and 4. The microbial data from different spots (* and **) were averaged and reported for the count of each site. Due to class schedules, the meat processing operation was not continuous from slaughtering till the final point of the meat fabrication. In the small scale operation, slaughtering beef cattle, pigs, and sheep was usually conducted every Tuesday and further steps of the operation for research or teaching purposes took place during the rest of the week.

The sampler used to impact the microorganisms from air was the Surface Air Sampler (SAS, Pool Bioanalysis Itliana, Milano, Italy, Figure 8). This sampler is relatively new portable battery operated unit which can be used in monitoring microbiological quality of air in hospitals, pharmacies, and food plants. The function of SAS is to aspirate air at a fixed rate, 3 Liters/second, through a cover which has been designed with small holes which direct the air flow onto


(Site 3)
(Site 1)
2/3
2/1
2/2
(Site 4)

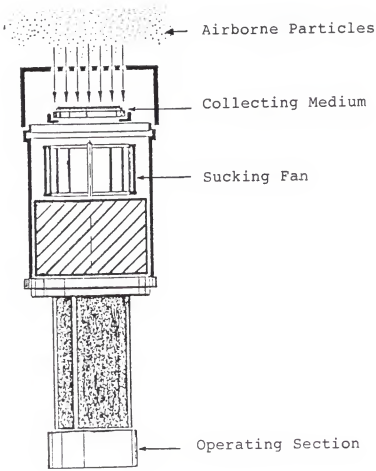


Figure 8:
Surface Air Sampler.

(Ligugnana, 1978)

a plate which contains a suitable medium. The amount of air that could be sucked through the unit range from 60 Liters/20 second to 900 liters/5 minutes. The desired volume of air to be tested can be chosen by a 15-unit knob attached to the system.

Temperature and humidity guages (Scientific Device Laboratory, Inc., Glenvlew, IL.) were used to monitor the temperature and the humidity in each site at the time of sampling. It took 15 min for the humidity guage to equilibrate at each site.

Ethyl alcohol was applied as a sanitizer to kill the microorganisms attached to the SAS cover from the previous sampling operation.

Plate count agar (PCA, Difco) was the medium used in recovering air microbes. Between 13.5 - 15 ml of the PCA was prepared and poured into the 65 x 15 mm-Rodac plates (Replicate Organisms Detection and Count, Becton Dickinson and Company; Oxnard, CA.) 3 -5 days before each sampling period. Violet Red Bile Agar (VRB) was used as a selective medium for gram-negative bacteria recovery.

Sampling Protocol

To study before and after occupancy, air in the meat laboratory complex was sampled in two distinct time periods (fourteen weeks each); the first period was before the occupancy when no operation related to meat processing was started and the second period was after the researchers and students had started meat processing. Dust, open rooms, and construction materials were the predominant elements in the first period. Different conditions including continuous cleaning, controlled temperature and air flow, increased number of people with different types of activity existed in the period after beginning operation of the meat processing facilities.

The air of the different sites was sampled once a week. One unit with SAS (60 Liter/20 Seconds) was the fixed rate to be tested from each position in each site for the Airborne Plate Count (ABPC). Before occupancy VRB agar was used to detect gram negative cells on the 5th, 7th, 10th, and 14th week and after occupancy on the 16th, 18th, 24th, and 27th week. The air samples were taken (sucked downward) at a 4.5 - 5 ft. height. Alcohol (95%) was applied after each sampling operation to sanitize the SAS cover. The temperature, humidity, number of people and their activities, and the presence of meat were always recorded.

To study microbial loads before, during, and after slaughtering operations, air of the slaughtering (from five locations at 4.5 - 5 ft. height) room and one cooler (2/3)

was sampled for 8 weeks. Every Tuesday, the samples were taken before the slaughtering operation, two times during the operation (2 hours apart), and a final sample taken 2 - 3 hours after cleaning the slaughtering room. The sample from the cooler (site 2/3) was usually taken as soon as the carcasses were moved inside. Beside the one unit used for testing ABPC, two units (120 Liters/ 40 seconds) were selected for the Coliform Count (ABCC). After each sampling operation the ABPC plates were incubated at 32 C for 48 hours and the VRB plates at 32 C for 24 hours before colonies were counted. Data from different spots of the same site were averaged. The airborne mold counts (ABMC), which were differentiated from bacteria due to colony morphologies, were obtained along with the ABPC obtained from PCA incubated at $32^{o}C$ for 48 hours.

Calculation and Statistical Analysis of The ABPC.

The ABPC of microbes obtained from 60 liters of air onto one plate containing PCA was converted to ABPC per cubic meter as follows:

$$\frac{\text{\# of microbes per sucked volume (liters)}}{\text{60 liters x unit (60 liter/20 Second.)}} \times 1000 = CFU/m^3$$

Descriptive analysis was used for the total airborne counts obtained from sites other than slaughtering room. A multiple comparison analysis was used to compare the ABPC before, during, and after the slaughtering operation (Snedecor and Cochran, 1967).

Identification Protocol

For identification of isolates, as many as eight colonies were randomly selected from Rodac plates and transferred with sterilized toothpicks into the microtiter wells containing PCA. Sterile Microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA.) were used to stock isolated colonies for identification. Each 96-well plate contained 3 drops of PCA per well. At this point, each isolated colony is presumed to be pure culture. At a later stage of analysis, gram staining procedure will reveal purity of isolates. The plates were then incubated at room temperature for 24 hours for colonies to grow and then refrigerated for further steps in identification of unknowns. About 24 hours before starting the identification process, brain heart infusion broth (two drops) were added to each microtiter well containing isolated cultures. The plates then were incubated at the room temperature for 24 hours to activate the cultures. After the incubation period, cultures from this Microtiter plate were transferred to another Microtiter plate containing PCA and kept as a reference.

With a multi-inoculator (Fung and Hartman, 1975), all 96 cultures (one from each well of the Microtiter plate) were transferred and smeared onto four neighboring microscopic slides (Figure 9) simultaneously. The cultures were heat-fixed and gram-stained. Gram morphology of microorganisms were obtained using the light microscope under oil immersion. The time of examining all four slides (96 cultures) usually

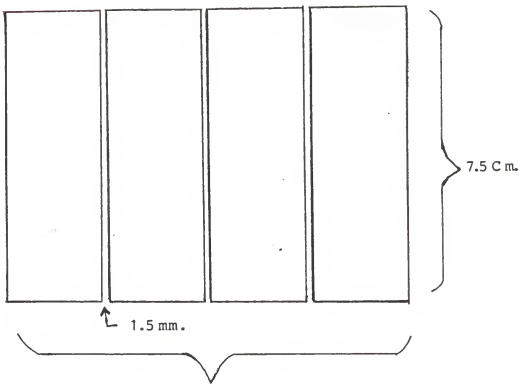


Figure 9:

Plain Slides conformation used in Gram Staining.

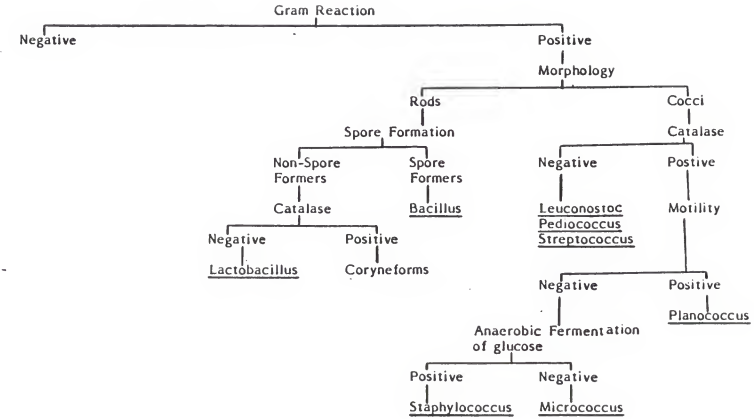
took from two to three hours. This procedure provided information of isolates concerning purity and gram morphology (positive or negative, rod or coccus). Yeast cells can also be recognized. Biochemical tests data were used only for pure cultures. Descriptive characterization of bacteria to genus level were made with the scheme (Figure 10) described by Gailani (1985), who obtained information from Buchanan and Gibbon (1974).

Motility test (motility agar, Difco) and the catalase test were also conducted in Microtiter plates. Methyl Red Agar was used to test the anaerobic fermentation of glucose.
From the original Microtiter plate, cultures were stabbed into another Microtiter plate containing Motility Agar (Difco) with a 96-pinpoint multi-inoculator. The motility results were read after incubation for 48 hours at 37°C with the help of a magnifying glass.

The Catalase test was conducted by placing one drop of 3% hydrogen peroxide (Fisher Scientific Company, St. Louis, MO) into each well containing reactivated isolates. Formation of bubbles indicate positive catalase test.

The Anaerobic Fermentation of Glucose was conducted to differentiate between Staphylococcus and Micrococcus. The nonmotile, gram positive, and catalase positive microorganisms were individually stabbed in tubes containing Methyl Red Agar

Characterization of Aerobic Bacteria Isolated from Dried Meat



(with added glucose). Mineral oil was added to each tube and then incubated the tubes at 32°C fore 48 hours. Staphylococcus provided positive test (a yellow tube indicates fermentation) and Micrococcus gave negative test (no color change indicates lack of fermentation). The mold counts (observation of mycelium and sporulation) were obtained along with the total microbial counts grown on PCA at 32°C for 48 hours.

Results and Discussion

The determination of the microbial profile before the operation of the new facilities in the meat processing laboratory at Kansas State University was an excellent chance to provide a good background of the microbial populations in the air of the facilities. The information which includes the type, number of microorganisms, and the condition influencing their presence can be used to compare with similar data after the facilities are used for teaching and research in meat processing.

In analyzing the data obtained, the total microbial count and mold count were plotted against time in weeks. A ranking system was designed to categorize the microbial counts in air before and after the occupancy. The designated ranges were: 1) <100 CFU/m^3, low count; 2) >100 - 300 CFU/m^3, intermediate count; and 3) >300 CFU/m^3, high count. The three ranges were based on the descriptive analysis accompanied the sampling operation throughout the 28-week period. It was found that whenever the air sample was taken from a clean site, with low temperature, less human activity, ventilation, and short stay of the meat, the total microbial counts were in the range of 100 CFU/m^3 or lower. More activity (mostly meat processing) with higher numbers of people and less cleanliness than the low range resulted in moderate microbial counts that ranged

between 101 and 300 CFU/m^3. Higher numbers than 300 CFU/m^3 were associated with presence of dust, high number of people (9 - 20), and live animals. These three ranges are applicable to the meat research complex at KSU or similar meat processing plants. Different ranges can be set in a similar way in a meat processing plant with different conditions or other food processing plants.

Analysis of ABPC at Site 1 (Teaching Laboratory)

The microbial, temperature, and humidity profiles of site 1 (Teaching laboratory) are presented in (Figure 11). In the first period (the last fourteen weeks of construction of the facilities) of this study, the temperature was in the range between 23 - 26°C. The relative humidity (R.H.) were between 54 and 75%. The ventilation system was not in operation (at all sites) throughout the first period (except in the second week for equipment testing). More dust and construction personnel were in the facilities during the first eleven weeks of the first period compared with week 12th, 13th, and 14th when preparations were started for occupancy. The ABPC at site 1 ranged from 37 CFU/m^3 in the thirteenth week, when constructing personnel started to move their tools and wash the floor, to 580 CFU/m^3 in the first weeks of sampling. In the second period of this study (weeks 15th to 28th), temperature at site 1 declined to 10 -21°C, ventilation was operative, and continuous cleaning was initiated, consequently

the airborne microbial counts dramatically declined. This site was also the least frequently used site after occupancy.

Figure 12 shows the changes in the % of samples which fell in each of the three microbial ranges before and after the occupancy of site 1. Before occupancy, the number of samples in each range was similar. After occupancy, however, a large % of samples fell in the low range and a few in the intermediate range whereas none was found in the high range.

Table 2 indicates the total airborne counts along with the existing conditions at the time of sampling at site 1.

Sites 2/1, 2/2, and 2/3 (Coolers) had the same conditions in the first period of air sampling. The temperature ranged from 22 to 26°C and R.H. from 54 to 75%. Some dust, construction tools, and people (8 or less) existed at some sampling periods. The ABPC in these sites were in the range of 110 - 530 CFU/m^3 which were relatively high.

In the second period (after occupancy) of sampling, the three coolers had different conditions which affected the microbial profiles.

Analysis of ABPC at Site 2/1 (Cooler)

In site 2/1, the temperature was in the range between 0 and 6°C. The R.H. ranged from 58 to 72%. Due to higher frequency of the presence of meat carcasses at this site

Figure 11:

The Microbial, Temperature, and Relative Humidity Profiles Before and After Occupancy at Site 1 (Teaching lab.).

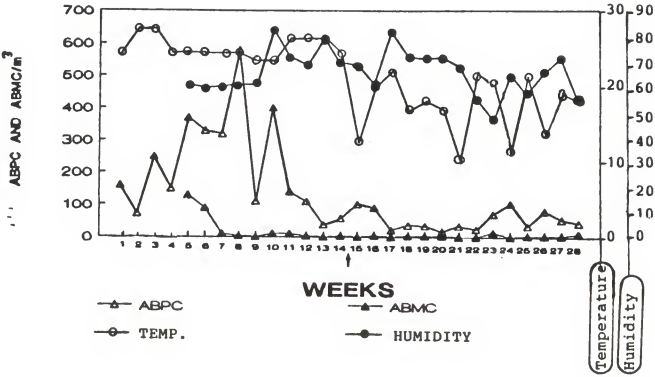


Figure 12:

The Changes in The Microbial Ranges Before and After Occupancy at Site 1 (Teaching Laboratory).

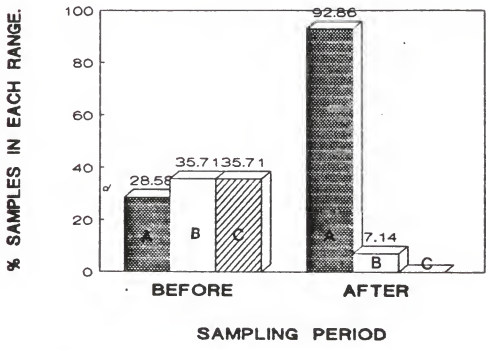


Table 2: Total Airborne Counts and the Existing Conditions at the Time of Sampling at Site 1 (Teaching Laboratory).

CFU/m^3	Temp. (°C)	Rela. Humi.	Condition(s)
1) ≤100 CFU/m^3 Range			
a 17	16	65	Clean, closed, none inside.
a 20	21	75	As above.
a 23	20	50	As above.
a 33	17	65	As above.
a 33	10	62	As above.
a 33	20	52	One person.
a 35	16	65	As above, water washed.
b 37	25	72	Clean, closed, none inside.
a 43	17	50	Clean, open.
a 53	18	65	As above.
b 57	23	63	As above.
a 70	20	42	Door was open, clean.
b 72	26	NP	Vent. Sys. was started, 7 people.
a 80	13	60	Clean, 4 people cutting meat.
a 90	10	56	As above, 5 students cutting meat.
b 100	25	62	Clean, none inside.
a 100	22	62	As above, cooling system started.
2) >100 - 300 CFU/m^3 Range			
a 103	11	59	Clean, open.
b 110	22	55	As above.
b 140	25	65	As above.
b 150	23	NP	Cleaner than above.
b 160	23	NP	Some dust, Vent. Sys. was off.
b 250	26	NP	As above.
3) >300 CFU/m^3 Range			
b 320	23	45	Dust coming from outside.
b 330	23	54	As above.
b 370	23	55	Four people, some dust.
b 400	22	75	Nine workers, open door.
b 580	23	55	Eight people, dirt on the floor.

a: After occupancy. b:Before occupancy.
NP: Not Preformed.

(2/1) during the sampling period compared with coolers 2/2 and 2/3, higher microbial counts were obtained. The total airborne counts ranged between 33 and 340 CFU/m^3, but 57.14% of the samples were higher than 100 CFU/m^3. This result indicates that the presence of the meat cuts for relatively long time (3 - 5 days) affected the microbial load in air of the cooler. With the exception of the 5th and the 6th week the mold count in the second period of site 2/1 is similar to the results obtained in the first period (before occupancy) where more than 90% of the samples had lower than 5 CFU of mold/m^3. Figure 13 shows the microbial, temperature and humidity profiles in both periods (before and after occupancy).

Figure 14 indicates the changes in the microbial ranges before and after occupancy at site 2/1. Before occupancy, most samples were in the intermediate to high microbial ranges whereas after occupancy more samples fell in the low microbial range category.

Table 3 shows the total airborne counts along with the existing conditions at the time of sampling at sit 2/1.

Analysis of ABPC at Site 2/2 (Cooler)

In site 2/2 (cooler), the temperature readings were below 4.5°C for all samples in the second period. The R.H. was in the range between 62 and 75%. This cooler was the least used

Figure 13:

The Microbial, Temperature, and Relative Humidity Profiles Before and After Occupancy at Site 2/1 (Cooler).

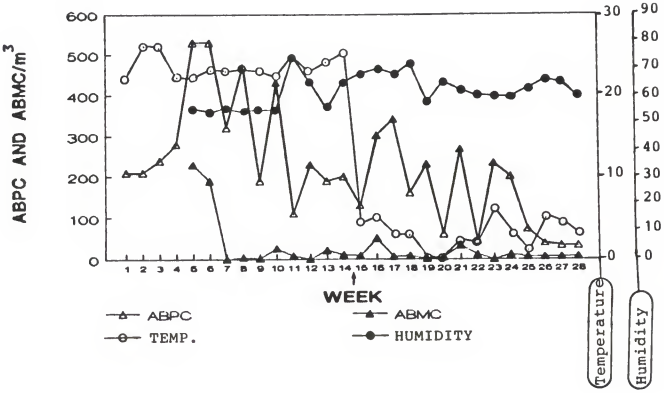


Figure 14:

The Changes in The Microbial Ranges Before and After Occupancy at Site 2/1 (Cooler).

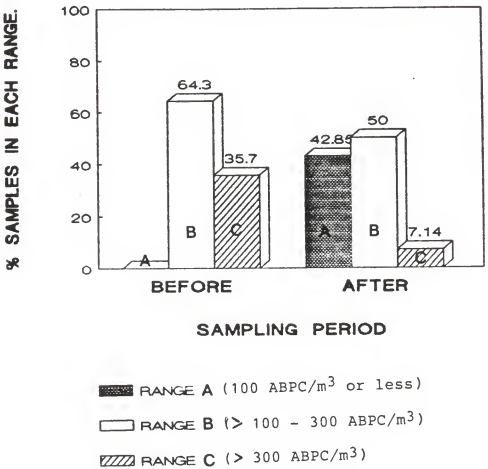


Table 3: Total Airborne Counts and the Existing Conditions at the Time of Sampling at site 2/1 (Cooler).

	CFU/m^3	Temp. (°C)	Rela. Humi.	Conditio(s)
1) ≤100 CFU/m^3 Range				
a	33	4.5	65	One pig on the rail.
a	33	03	60	Three pigs.
a	39	03	66	No animal was hanged, was open to 2/2
a	72	01	63	One & 1/2 cattle, 1 pig, ground meat.
a	44	02	60	Washed 5 days before, 4 cattle.
a	60	00	65	One beef carcass on the rail, clean floor.
2) >100 - 300 CFU/m^3 Range				
b	110	25	74	Clean, closed, none inside.
a	130	4.5	68	Clean, cooling system was started.
a	160	03	72	Four cattle, 2 pigs.
b	190	23	55	Closed, cleaner than above.
b	190	24	56	Clean, open.
b	200	25	65	Closed, clean.
a	200	03	60	Four pigs, some blood on the floor.
b	210	22	NP	Vent. Sys. was off, little dust.
b	210	26	NP	Vent. Sys. was on, " " .
b	230	23	65	Clean, four workers.
a	230	00	58	Fourteen pigs, 4 cattle.
a	233	06	60	Three cattle, blood on the floor.
b	240	26	NP	Dirt on the floor, V.S. was off,
a	267	02	62	Three pigs, 2 cattle.
b	280	22	NP	As above.
a	300	05	70	Three cattle, blood on the floor.
3) >300 CFU/m^3 Range				
b	320	23	55	Four workers, some dust.
a	340	03	68	Empty, but blood on the floor.
b	430	22	55	Little dust, nine workers.
b	470	23	54	Little dust, 4 - 6 workers.
b	530	22	55	Tow workers, tools, dust.
b	530	23	54	Dust, 4 - 6 workers.

a: After occupancy.
b: Before occupancy.
NP: Not preformed

for meat chilling. At this site, 80% of the samples had lower than 100 CFU/m^3. The mold counts were less than 17 CFU/m^3 in 95% of the samples. Figure 15 shows the microbial, temperature, and R.H. profiles at site 2/2.

Figure 16 indicates the changes in the microbial ranges occurred before and after occupancy at site 2/2. Before occupancy all samples were higher than 100 CFU/m^3 whereas after occupancy most of samples were in the low microbial range.

Table 4 shows the total airborne counts along with the existing conditions at the time of sampling at site 2/2.

Analysis of ABPC at Site 2/3 (Cooler)

The human activity and total microbial count at site 2/3 (Cooler) were between those observed and collected at the sites 2/1 and 2/2. With the exception of the 24th week, the temperature readings were 4.5°C or less. The R.H. was in the range between 65 and 75%. This cooler was always used to receive the carcasses after slaughtering operation. Figure 17 shows the microbial, temperature, and R.H. profiles in site 2/3.

Figure 18 indicates the changes in the microbial ranges occurred before and at occupancy at site 2/3. Before occupancy the microbial counts were all above 100 CFU/m^3, but after occupancy most samples (78.57%) fell in the low microbial range.

Figure 15:

The Microbial, Temperature, and Relative Humidity Profiles Before and After Ocupancy in Site 2/2 (Cooler).

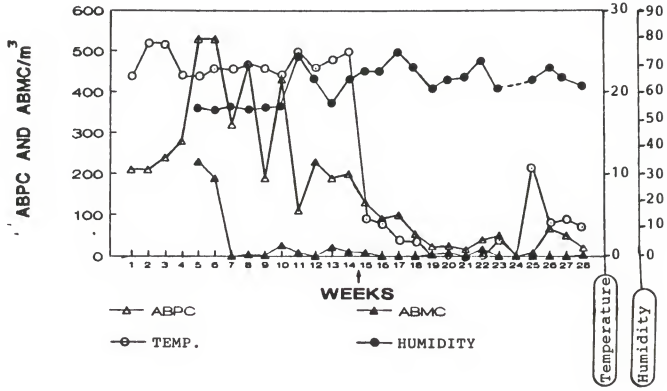


Figure 16:

The Changes in The Microbial Ranges Before and After Occupancy at Site 2/2 (Cooler).

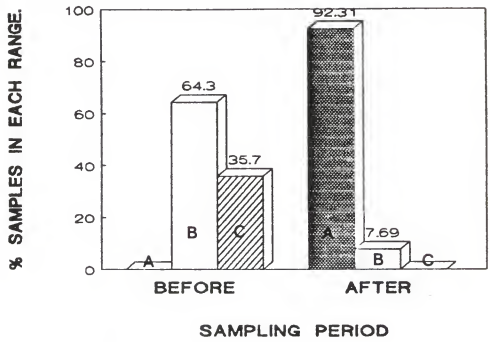


Table 4: Total Airborne Counts and the Existing Conditions at the Time of Sampling at Site 2/2 (Cooler)[1].

CFU/m^3	Temp. (°C)	Rela. Humi.	Condition(s)
1) ≤100 CFU/m^3 Range			
a 08	01	65	Empty, clean.
a 16	00	66	As above.
a 23	00	62	As above.
a 25	00	65	As above.
a 25	3.5	62	As above.
a 40	00	72	As above.
a 50	02	62	As above.
a 50	4.5	65	As above.
a 54	02	70	As above.
a 67	04	69	Little blood on one corner, no meat.
a 92	04	68	Empty, clean.
a 100	02	75	Seven pigs.
2) >100 - 300 CFU/m^3 Range			
a 130	4.5	68	None was inside, (15 th week).

a: After occupancy.

[1] The total airborne counts and the conditions before occupancy are the same as the ones in site 2/1. (Notice: The site was closed in the 24 th week)

Figure 17:

The Microbial, Temperature, and Relative Humidity Profiles Before and After Ocupancy at Site 2/3 (Cooler).

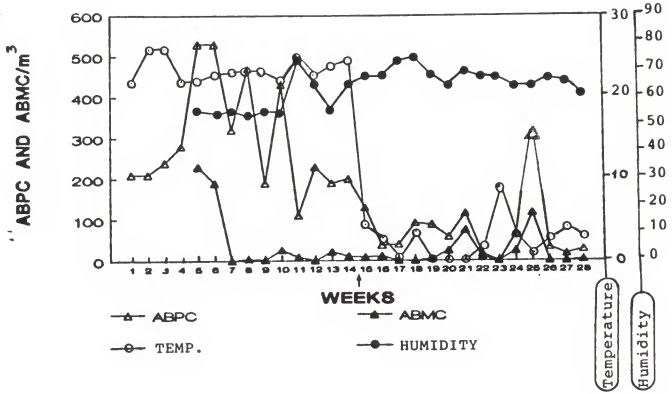


Figure 18:

The changes in The Microbial Ranges Before and After Occupancy at Site 2/3 (Cooler).

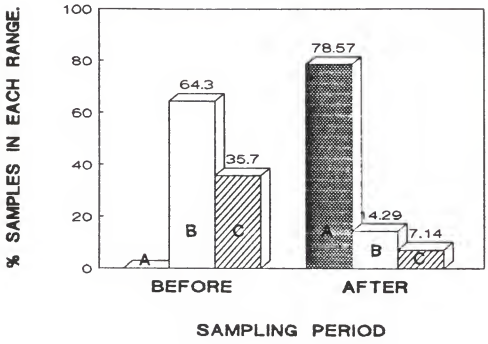


Table 5 indicates the total airborne counts along with the existing conditions at the time of sampling in site 2/3

Table 5: Total Airborne Counts and the Existing Conditions at the Time of Sampling in Site 2/3 (Cooler)[1]

CFU/m^3	Temp. (°C)	Rela. Humi.	Condition(s)
1) ≤100 CFU/m^3 Range			
a ND	09	68	Clean, empty, closed.
a 17	02	68	As above.
a 17	04	66	As above.
a 28	03	62	Six pigs hanged the day before.
a 33	03	68	Five cattle just hanged.
a 38	03	68	Empty, clean.
a 40	00	74	As above.
a 92	3.5	75	As above.
a 58	00	65	As above.
a 67	03	65	Four pigs, little blood.
a 88	00	69	One cattle just hanged.
2) >100 - 300 CFU/m^3 Range			
a 116	00	70	Clean, empty.
a 130	4.5	68	As above.
3) >300 CFU/m^3 Range			
a 358	03	65	Empty, open to the slaughtering room

a : After occupancy.

[1] The Total airborne counts and the conditions before occupancy are the same as the ones in site 2/1.

Analysis of ABPC at Site 3 (Meat Fabrication Laboratory)

The conditions at site 3 (Meat fabrication laboratory) presented in the first period were similar to the ones in the other sites. The temperature was in the range between 22 and 26°C and the R.H. between 54 and 73%. Dust and number of people were similar to those in site 1 except in the 12th week when more people and equipment were present in site 3. The total airborne counts in the first period ranged between 107 and 833 CFU/m^3 in the first 12 weeks, but lower numbers (73 and 87 CFU/m^3), in the 13th and 14th weeks as results of clean-up operations. In the second period of air sampling, the temperature at site 3 declined and remained at the level between 6 and 13°C. The R.H. ranged from 55 to 78%. During most of the sampling periods from 3 to 7 people were found working with meat cuts. About 60% of the samples had total microbial counts less than 100 CFU/m^3, and the rest of the samples were in the intermediate range (>100 - 300 CFU/m^3). The mold count was similar to the ones in the first period except in the 6th and 7th weeks. Figure 19 shows the microbial, temperature, and R.H. profiles in site 3.

Figure 20 indicates the changes in the microbial ranges that occurred before and after occupancy of site 3. Before occupancy all samples were above 100 CFU/m^3 whereas after occupancy most of the microbial counts were in the low and intermediate ranges.

Figure 19:

The Microbial, Temperature, and Relative Humidity Profiles Before and After Occupancy at Site 3 (Meat Fabrication Room).

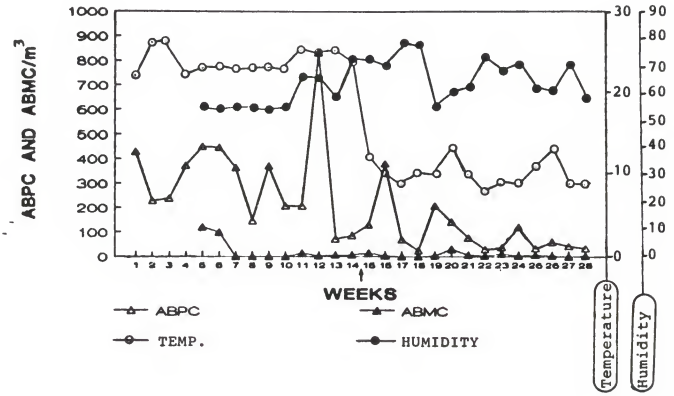


Figure 20:

The Changes in The Microbial Ranges Before and After Occupancy in Site 3 (Meat Fabrication Room).

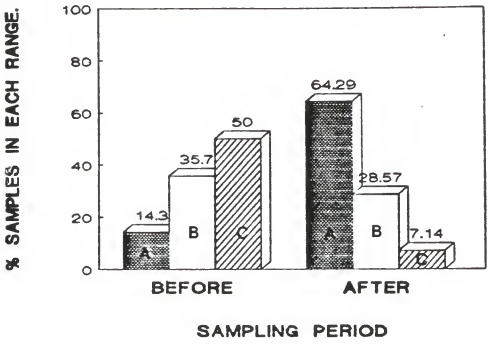


Table 6 shows the total airborne counts along with the existing condition at the time of sampling in site (3).

Analysis of ABPC at Site 4 (Abattoir)

The last part of this study involved in analysis of airborne microorganisms in the abattoir as well as in site 2/3 which was the receiving cooler for the carcasses. In the abattoir, air samples were monitored only after occupancy of the complex; the conditions and the activity were different from the ones in the other sites. The temperature before, during, and after operation ranged from 12 to 21^{o}C, and the R.H. ranged from 30 to 86%. The higher R.H. readings were recorded during the slaughtering operation due to the use of water and boiling water. The site was always rinsed, soaped, and rinsed after slaughtering. During operation, animals including cattle (1 - 3), pigs (3 - 6), and/or sheep (2 - 4) as well as from 9-20 students and personnel were in the slaughtering area. Table 7 shows the total airborne count along with the existing conditions at each sampling period. A multiple comparison procedure was used to ascertain if there was a significant difference in the total airborne count before (31 CFU/m^3), hour 0 (796 CFU/m^3 at the begining of operation), hour 2 (564 CFU/m^3, two hours after operation), and after the slaughtering operation (56 CFU/m^3). There was no significant difference ($P< 0.05$) in the total airborne counts between before and after slaughter samples

Table 6: Total Airborne Counts and the Existing Conditions at the Time of Sampling at Site 3 (Meat Fabrication Room)

CFU/m^3	Temp. (°C)	Rela. Humi.	Condition(s)
1) ≤100 CFU/m^3 Range			
a 25	10	78	Empty, clean.
a 30	08	74	As above.
a 33	11	62	Five people working with meat.
a 33	09	58	Empty, clean.
a 37	09	68	One person cutting meat.
a 43	09	70	Three people.
a 60	13	60	Four people.
a 70	09	78	Empty, clean.
b 73	25	58	As above.
a 77	10	62	Open, clean, 6 - 8 people.
b 87	24	73	Empty, clean.
2) >100 - 300 CFU/m^3 Range			
b 107	23	54	None was inside, closed.
a 120	09	71	Seven people cutting meat.
a 130	12	72	Vent. Sys. ON, clean, closed.
a 143	13	60	Four people, clean.
b 170	23	55	Eight workers.
a 207	10	55	Empty, clean.
b 207	25	66	Some shelves, clean.
b 229	26	NP	Vent. Sys. was ON, little dust.
b 239	26	NP	Vent. Sys. Little dust.
3) >300 CFU/m^3 Range			
b 363	23	55	Had some dirt.
b 370	23	55	Nine workers, open door.
b 372	22	NP	Had some dirt.
a 380	10	70	Two carcasses, 2 students.
b 429	22	NP	Dust, tools, Vent. Sys. was OFF.
b 447	23	54	Had some dirt.
b 450	23	55	As above.
b 833	25	65	Had some shelves,clean, 9 workers.

a: After occupancy.
b: Before occupancy.
NP: Not preformed.

Table 7: Total Airborne Counts and the Existing Conditions at the Time of Sampling at Site 4 (Abattoir)

CFU/m^3	Temp. (°C)	Rela. Humi.	Condition(s)
1) ≤100 CFU/m^3 Range			
as 17	17	60	Empty, clean.
bs 17	19	44	As above.
bs 20	18	50	As above.
bs 20	17	50	As above.
as 23	18	60	As above.
bs 27	17	58	As above.
bs 27	16	55	As above.
bs 27	18	45	As above.
as 33	14	45	As above.
bs 33	17	55	As above.
bs 37	13	55	As above.
bs 53	19	35	As above.
as 61	17	45	As above.
as 63	17.5	65	As above.
as 63	20	30	As above.
2) >100 - 300 CFU/m^3 Range			
as 158	18.5	48	Sample taken soon after washing.
ds 247	15	62	Two cattle, 8 people.
ds 287	21	40	Nine people, six pigs.
3) >300 CFU/m^3 Range			
ds 517	15	65	Two cattle, 20 students.
ds 770	15	68	End of slau. operation, 20 students.
ds 833	12	65	Three pigs, 16 people.
ds 925	17	55	Four cattle, 16 students.
ds 367	17	60	Three sheep, 19 people.
ds 567	20	60	Ten people, 2 pigs.
ds 793	19	52	Nine people, 2 pigs.
ds 892	21	53	Ten people, six pigs
ds 1630	17	58	Two sheep, 3 pigs, 20 students.

bs: Before slaughtering and cleaning.
ds: During slaughtering.
as: After slaughtering.

(31 CFU/m^3 vs. 56 CFU/m^3), and between hour 0 and hour 2 samples (796 CFU/m^3 vs. 564 CFU/$m^{3)}$. A significant difference (P>0.05), however, was found between the total count during slaughtering (hour 0, 796 CFU/m^3; and hour 2, 564 CFU/m^3), versus before (31 CFU/m^3) or after (56 CFU/m^3) slaughtering.

We concluded that the total airborne counts were higher during slaughtering than before or after slaughtering (Table 8). The airborne mold counts were similar in all sampling periods, and airborne coliform bacteria were only detectable during slaughtering. These findings indicate that live animals entering the facilities were responsible for the presence of coliform bacteria as well as the high counts of airborne microbes during the slaughtering operation.

The determination of the total airborne count in site 2/3 (Cooler) after receiving the carcasses from the abattoir showed similar results (87.5% of the samples had lower than 100 CFU/m^3) to the samples (87.57% of the samples had lower than 100 CFU/m^3) taken at the beginning of this investigation (the second 14 weeks of the initial sampling of this site). This result indicates that after the slaughtering operation, effective water-jet cleaning of the carcasses before sending the meat to the cooler (2/3) reduce microbial contamination of the air in the cooler. About 25% of the samples (8 samples) had coliform bacteria on VRB medium. The mold count did not exceed 17 CFU/m^3.

Table 8: The Total Airborne, Mold, and Coliform Counts[1]

Before, During, and After Slaughter.

Week	Counts	Before Slaught.	Hour 0	Hour 2	After Slaught.
1	ABPC	37	517	770	17
	ABMC	07	010	033	ND
	ABCC	ND	008	ND	ND
2	ABPC	33	247	833	23
	ABMC	03	010	008	ND
	ABCC	ND	ND	004	ND
3	ABPC	27	925	367	63
	ABMC	07	004	ND	07
	ABCC	ND	004	ND	ND
4	ABPC	53	567	NP	33
	ABMC	17	023	NP	13
	ABCC	ND	ND	NP	ND
5	ABPC	30	1630	NP	27
	ABMC	07	020	NP	ND
	ABCC	ND	004	NP	ND
6	ABPC	17	NP	287	63
	ABMC	ND	NP	010	07
	ABCC	ND	NP	004	ND
7	ABPC	27	793	NP	158
	ABMC	ND	003	NP	33
	ABCC	ND	008	NP	ND
8	ABPC	20	892	NP	61
	ABMC	03	ND	NP	ND
	ABCC	ND	004	NP	ND

[1] Total numbers per m^3.
Hour 0 = At the beginning of slaughtering operation.
Hour 2 = After 2 hours of slaughtering operation.
ABPC: Airborne Plate Count.
ABMC: Airborne Mold Count.
ABCC: Airborne Coliform Count.
NP: Not preformed, ND= Not detectable.

Table 9: The Total Airborne, Mold, and Coliform Counts[1] After Receiving the Carcasses in Site 2/3 (Cooler)

Week	ABPC	ABMC	ABCC
1	50	ND	4
2	33	ND	ND
3	125	ND	4
4	92	17	ND
5	42	ND	ND
6	17	ND	ND
7	92	8	ND
8	75	ND	ND

ABPB: Airborne Plate Count.
ABMC: Airborne Mold Count.
ABCC: Airborne Coliform Count.
ND: Not Detectable
[1] Total numbers per m^3

In general the conditions (temperature, R.H., type and intensity of activities, and cleanness) of all sites studied were similar in the first period (during construction). A decline of the microbial load was observed at the last few weeks of the first period due to the reduced construction activity and more clean-up operations.

The condition were dramatically changed in the second period of this study. The temperature declined in the coolers to less than 6°C, in the fabrication room to the range of 8 - 13°C, and in the teaching laboratory to the range of 10 - 21°C. The relative humidity increased especially in the coolers due to the continuous use of water and the presence of meat. The simultaneous presence of desirable factors including low temperature, frequent cleaning, ventilation,

and good handling of the meat cuts resulted in acceptable levels (< 100 ABPC/m^3) of the total airborne count. Although low temperature in a cooler usually resulted in lower microbial counts in the air, the results of this study indicates that site 2/1, which had conditions (including low temperature) similar to site 2/2 had higher microbial counts (153 ABPC/m^3 vs. 52 ABPC/m^3). This was because of the presence of carcasses in site 2/1 versus absence or short stay of carcasses in site 2/2.

In the abattoir house (site 4), most of the total airborne counts as well as the coliform counts were attributed to dirt, hair, and activities live animals.

Identification of Airborne Mcirobial Isolates

Another aspect of this investigation was to study the kinds of microorganisms in the air before and after occupancy of the facilities. The morphological, physical, and biochemical analysis of 728 microbial isolates from the first period (before occupancy) of the study showed that 38.9% were gram-positive cocci (most of which were Micrococcus), 56.6 % were gram-positive rods (most of which were Bacillus), and 4.5 % was mixed cultures. After occupancy microbial identification of 444 isolates indicated that 25 % gram-positive cocci (most of which were Micrococcus), 46.4 % were gram-positive rods (most of which were Bacillus), 18.5 % were yeasts, 3.6 % were mixed cultures, and 6.3 % were dead

cultures (no growth at the time of identification). Table 10 shows the different types of microorganisms (based on the identification scheme presented in the materials and method section). Identification of mold was not made in this survey study although mold counts were made.

It is noteworthy that gram-negative cells were not isolated from non-selective medium (Plate Count Agar) indicating that the number of gram-negative cells were much lower than gram-positive cells in air before and after occupancy of the facilities. ABCC data during the two periods also showed negative growth of gram-negative bacteria on VRB agar. Other investigators (Kotula and Emswiler-Rose, 1988) also presented similar data. The appearance of yeast (18.5%) after occupancy indicates that yeast in air was associated with meat. With the use of selective medium (VRB), coliform bacteria were found at the abattoir and in the samples taken from site 2/3 after receiving the carcasses. However, the numbers were low (5 and 4 ABCC/m^3). Molds were found before and after occupancy of the facilities at low levels (>99% of the samples were <100 ABMC/m^3)

Table 10: The Types of Airborne Microorganisms Found in the Meat Research Complex Before and After Occupancy.

M.O.	Before[1] (%)	After[2] (%)
1. Bacteria.		
A) Gram-positive-cocci.	38.88	25.10
a) Micrococcus.	25.44	18.69
b) Pediococcus, Leuconostoc, and/or Streptococcus.	7.01	2.25
c) Staphylococcus.	6.43	4.27
B) Gram-positive-rods.	56.59	46.50
a) Bacillus.	43.96	31.53
b) Coryneforms.	10.85	13.06
c) Lactobacillus.	1.78	1.8
2. Yeast.	00	18.50
3. Mixed cultures.	4.53	3.60
4. Dead cultures.	00	6.30

[1] Based on 728 microbial isolates from samples obtained before occupancy.

[2] Based on 444 microbial isolates from samples obtained after occupancy.

Summary

The high level of Airborne Plate Count (ABPC) in the first several weeks (in all sampled sites) of the first period (before occupancy) was the result of dust along with intensive construction work, and no cleaning or ventilation. The ABPC started to decline in the last few weeks of the first period (before occupancy) as a result of less construction work, more cleaning, and operation of the ventilation system.

In the second period of this investigation (after occupancy), a general decline in the ABPC occurred in all sites as the temperature decreased, ventilation was in operation, and frequent cleaning took place. The highest ABPC among these sites was in site 2/1 (cooler) as results of the relatively longer time in which carcasses were retained.

The type of activity (slaughtering) in the abattoir increased the number of airborne microorganisms during slaughter whereas much lower airborne counts were obtained before and after slaughter.

The decline of the total airborne counts as the carcasses were further processed (slaughtered, chilled, fabricated) was similar to results reported from a pork processing plant (Kotula and Emswiler-Rose, 1988). The total airborne counts obtained at KSU meat research complex ,however, were much lower compared with the intensive production (slaughtering at 380 hogs per hour) in the pork processing plant (52 - 3.1 x

10^2 ABPC/m^3 vs. 3.2 x 10^2 - 1.1 x 10^4 ABPC/m^3) The pattern (decline of ABPC as the carcasses are further processed) has not been found only in meat processing plants , but also in poultry processing plants (Kotula and Kinner, 1964). The absence of airborne coliform bacteria in the air samples from sites other than the slaughtering room and cooler 2/3 (only after receiving the carcasses) has been found also in another investigation by Kotula and Emswiler-Rose (1988).

The ranking system ($\leq$100, >100 - 300, and >300 CFU/m^3) used to categorize the airborne microbial counts in this investigation can be adopted to meat (food) processing conditions similarly to the ones at KSU meat research laboratory complex or changed occordingly after similar investigation (Ligugnana, 1982) . Similar ranking system have been suggested to monitor the airborne microbial counts in different environments whenever the SAS is used. The mean of airborne counts in food processing plants was suggested to be between 15 to 75 CFU/m^3 (Ligugnana, 1982).

The identification of more than 1,200 microbes obtained before and after occupancy showed that Micrococcus and Bacillus were the most predominant microorganisms indicating a high sustainability under various conditions. The coliform bacteria were found in air in the slaughtering room when live animals were introduced. Yeast appearance after occupancy of

facilities may be associated with the presence of meat animal and meat products.

Conclusions

1) Dust from building construction was the major source of airborne microorganisms in the first period of sampling (before occupancy) of the meat laboratory complex.
2) The simultaneous presence of favorable factors after occupancy such as low temperature, cleanliness, ventilation, and few people were essential in reducing the air borne microbes in the KSU meat laboratory complex.
3) Live animals and slaughtering operation contributed to the highest airborne microbial counts compared with other sources in the facilities after occupancy.
4) Higher airborne microbial counts were obtained in cooler 2/1 where carcasses stayed longer (3 - 5 days) than cooler 2/2 and 2/3 where carcasses stayed shorter (one day or less).
5) Proper processing conditions during and after the slaughtering of animals resulted in acceptable number (<100 CFU/m^3) of microbes in the cooler (site 2/3) where the carcasses were received.
6) Violet Red Bile Agar data indicated that in the slaugh tering room and in cooler 2/3, at the time of receiving the carcasses, were gram negative bacteria in the air whereas no gram negative bacteria were detected at other sites.

7) The presence of yeast was associated with the presence of meat

8) The most predominant microorganisms in air in this study were Micrococcus and Bacillus.

9) The designated microbial ranges ($\leq$100 ABPC/m^3, low; >100 - 300 ABPC/m^3, intermediate; and >300 ABPC/m^3, high) are applicable only at KSU meat research complex and/or other meat processing plants with similar conditions.

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Appendixes

Appendix 1:

Microbial[1], Temperature, and Humidity Profiles at Site 1.

(Teaching Lab.)

Time (Weeks)	Total Count	Mold Count	Temp.	Humi.
1	160	NP	23	NP
2	72	NP	26	NP
3	250	NP	26	NP
4	150	NP	23	NP
5	370	130	23	55
6	330	90	23	54
7	320	10	23	54
8	580	03	23	55
9	110	ND	22	55
10	400	10	22	75
11	140	10	25	65
12	100	03	25	62
13	37	ND	25	72
14	57	03	23	63
15	100	ND	12	62
16	90	03	10	56
17	20	ND	21	75
18	35	02	16	65
19	33	02	17	65
20	17	03	16	65
21	33	ND	10	62
22	23	ND	20	50
23	70	13	20	42
24	103	00	11	59
25	33	03	20	52
26	80	03	13	60
27	53	03	18	65
28	43	10	17	50

[1] Microbial Count Per Cubic Meter of Air.
ND: Not detectable.
NP: Not preformed.

Appendix 2:

Microbial[1], Temperature, and Humidity Profiles at Site 2/1. (Cooler).

Time (Weeks)	Total Count	Mold Count	Temp.	Humi
1	210	NP	22	NP
2	210	NP	26	NP
3	240	NP	26	NP
4	280	NP	22	NP
5	530	230	22	55
6	530	190	23	54
7	320	ND	23	55
8	470	04	23	54
9	190	02	23	55
10	430	26	22	55
11	110	08	25	74
12	230	ND	23	65
13	190	21	24	56
14	200	10	25	65
15	130	08	4.5	68
16	300	50	05	70
17	340	06	03	68
18	160	08	03	72
19	230	01	00	58
20	60	05	00	65
21	267	33	02	62
22	44	11	02	60
23	233	ND	06	60
24	200	11	03	60
25	72	06	01	63
26	39	06	05	66
27	33	06	4.5	65
28	33	07	03	60

[1] Microbial Counts Per Cubic Meter of Air (average of 5 locations).
ND: Not detectable.
NP: Not preformed.

Appendix 3:

Microbial[1], Temperature, and Humidity Profiles at Site 2/2. (Cooler).

Time (Weeks)	Total Count	Mold Count	Temp.	Humi
1	210	NP	22	NP
2	210	NP	26	NP
3	240	NP	26	NP
4	280	NP	22	NP
5	530	230	22	55
6	530	190	23	54
7	320	ND	23	55
8	470	04	23	54
9	190	02	23	55
10	430	26	22	55
11	110	08	25	74
12	230	ND	23	65
13	190	21	24	56
14	200	10	25	65
15	130	08	4.5	68
16	92	ND	04	68
17	100	ND	02	75
18	54	ND	02	70
19	23	04	00	62
20	25	08	00	65
21	16	ND	00	66
22	40	17	00	72
23	50	ND	02	62
24	NP	NP	NP	NP
25	08	ND	01	65
26	67	ND	04	69
27	50	ND	4.5	65
28	20	03	3.5	62

[1] Microbial Count Per Cubic Meter of Air (average of 2 locations).
ND: Not detectable.
NP: Not preformed.

Appendix 4:

Microbial[1], Temperature, and Humidity Profiles at Site 2/3. (Cooler).

Time (Weeks)	Total Count	Mold Count	Temp.	Humi
1	210	NP	22	NP
2	210	NP	26	NP
3	240	NP	26	NP
4	280	NP	22	NP
5	530	230	22	55
6	530	190	23	54
7	320	ND	23	55
8	470	04	23	54
9	190	02	23	55
10	430	26	22	55
11	110	08	25	74
12	230	ND	23	65
13	190	21	24	56
14	200	10	25	65
15	130	08	4.5	68
16	38	17	03	68
17	40	ND	00	74
18	92	ND	3.5	75
19	88	04	00	69
20	58	25	00	65
21	116	75	00	70
22	17	08	02	68
23	ND	ND	09	68
24	67	25	03	65
25	358	117	01	65
26	33	ND	03	68
27	17	ND	04	66
28	28	03	03	62

[1] Microbial Count Per Cubic Meter of Air (average of 3 locations).
ND: Not detectable.
NP: not preformed.

Appendix 5:

Microbial[1], Temperature, and Humidity Profiles at Site 3.

(Meat Fabrication Room).

Time (Weeks)	Total Count	Mold Count	Temp.	Humi
1	429	NP	22	NP
2	229	NP	26	NP
3	239	NP	26	NP
4	372	NP	22	NP
5	450	120	23	55
6	447	100	23	54
7	363	ND	23	55
8	170	ND	23	55
9	107	ND	23	54
10	370	ND	23	55
11	207	13	25	66
12	833	03	25	65
13	73	07	25	58
14	87	07	24	73
15	130	13	12	72
16	380	03	10	70
17	70	ND	09	78
18	25	ND	10	78
19	207	03	10	55
20	143	30	13	60
21	77	07	10	62
22	30	03	08	74
23	37	10	09	68
24	120	03	09	71
25	33	07	11	62
26	60	03	13	60
27	43	ND	09	70
28	33	03	09	58

[1] Microbial Count Per Cubic Meter of Air (average of 5 locations).

ND: Not detectable.

NP: Not preformed.

AEROMICROBIOLOGY: AN ASSESSMENT OF A NEW MEAT RESEARCH COMPLEX.

by

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Abstract

Airborne microorganisms at the Kansas State University meat research complex were enumerated before and after occupancy for twenty eight weeks (14 before occupancy and 14 after occupancy) using Surface Air Sampler (SAS). Unhygienic condition was predominant in the first 14 weeks due to construction of the building whereas improved conditions including continuous cleasing, ventilation, and lower temperature were present in the second period of the study (after occupancy). The sites where air was sampled were meat teaching laboratory (site 1), three neighboring coolers (sites 2/1, 2/2, and 2/3), and a meat fabrication room (site 3). The average weekly airborne plate counts (ABPC) before and after occupancy were 220 ABPC/m^3 and 52 ABPC/m^3, 296 ABPC/m^3 and 153 ABPC/m^3, 296 ABPC/m^3 and 52 ABPC/m^3, 296 ABPC/m^3 and 77 ABPC/m^3, and 313 ABPC/m^3 and 99 ABPC/m^3, for sites 1, 2/1, 2/2, 2/3, and 3, respectively, indicating a decline of airborne microbial counts after occupancy.

The air microbial quality in the slaughtering room was monitored only after occupancy. The airborne microorganisms in this site were enumerated before, during (0 hr. and 2 hr.), and after slaughtering operation for eight weeks. The average total airborne plate count was 796 ABPC/m^3 (0 hr.) and 564 ABPC/m^3 (2 hr.) during slaughtering operation which were much

higher than those obtained before (31 ABPC/m^3) and after (56 ABPC/m^3) slaughtering operation.

In general airborne mold counts (ABMC) at all sites (either before or after occupancy and before, during, or after slaughtering operation) were less than 20 ABMC/m^3. The coliform bacteria were detectable only during slaughtering operation and after receiving the carcasses in site 2/3 (cooler).

Airborne yeasts were found in the facilities only after meat was introduced indicating a possible role of yeast in meat microbiology.

The identification of 728 microbial isolates before occupancy and 444 isolates after occupancy indicated that most airborne bacteria were gram-positive rods (mostly Bacillus) and gram-positive cocci (mostly Micrococcus).